Life Cycle of Viruses

Introduction

Viruses are hijackers. They rely entirely on the cell they infect to do all the work. Viruses do not generate energy, so they rely on the cell to provide it. They rely on the cell to provide resources—nucleotides, amino acids, and sometimes a plasma membrane (for enveloped viruses). Some viruses even rely on the host cell to replicate the virus. We use this lesson to cover viral replication cycles, first generally, then in regard to DNA, ss(+)RNA, and ss(–)RNA viruses specifically. We do it this way so that once we finish the biochemistry and physiology of replication, we can focus on the diseases caused by viruses in subsequent lessons. We won't be writing out or drawing the life cycle of every virus. Instead, we want you learning life cycles in general, then apply the appropriate life cycle to the cluster of viruses that share that life cycle. We close this lesson with the cellular response to an attempted infection, then correlate the relationship of the virus and its host to the expression of disease.

Life Cycle in General

There are three main phases of viral replication: Get in, get it done, get out.

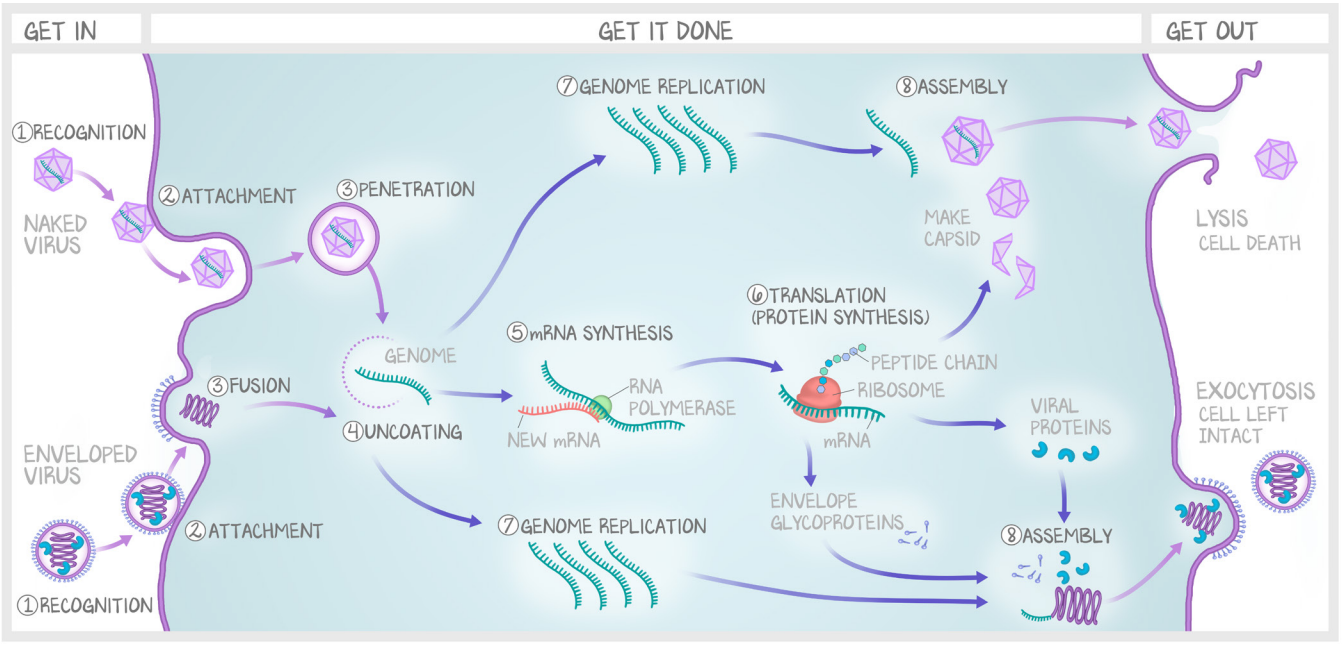

Figure 2.1: Generalized Viral Replication Scheme
This figure is meant to serve as a visual guide through the next several pages. While there are many steps involved in the viral life cycle, and dissimilarities between enveloped and naked viruses, the general theme remains the same—get in, get it done, get out. That is, the virus needs to get into the host cell (get in); manipulate that cell to replicate the viral genome, produce virion proteins (and in most cases, more complex viral proteins) (get it done); and then eventually leave to infect the next cell (get out).

"Get in" means recognition (1), **attachment** (2), **penetration/fusion** (3), **and uncoating** (4). The virus floats around doing nothing until it bumps into a cell it recognizes. Viral attachment proteins (VAPs) are structures on the outermost part of the virus—capsid for naked viruses, envelope for enveloped virus. VAPs interact with host cell membrane proteins, effectively viral receptors, to induce the membrane to let the virus in. These receptors are not made by the cell to be viral receptors, of course. Viruses hijack a normal, functioning cell membrane receptor to trick the cell into letting the virus in. Attachment and penetration go hand in hand—the thing the virus uses to attach, it uses to penetrate.

If that virus has an **envelope** (a plasma membrane), the attachment occurs between the glycoproteins of the envelope and the cell membrane of the target cell. Because viruses package themselves in a cell, then leave that cell wearing its plasma membrane as the envelope, the next cell of that type it encounters will recognize the membrane as friendly. When a vesicle carrying enzymes fuses with the plasma membrane, those enzymes are released, a process called exocytosis. That is achieved by fusing the membrane of the vesicle with the plasma membrane. Viruses do something similar. Enveloped viruses have cytoplasm inside the virus "vesicle" taken from the cell it budded from. Now the plasma membrane of the cell and the virus "vesicle" fuse, and the capsid is inside the cytoplasm of the cell.

If that virus is **naked** (no plasma membrane), then this fusion business cannot happen. Instead, **viral surface proteins** activate **transmembrane proteins** on the host cell, **inducing endocytosis**. How the virus gets from the endosome into the cytoplasm is too much detail to worry about. Suffice it to say, the virus activates its receptor, and ends up in the cytoplasm of the cell.

Uncoating is the release of the viral genome from the nucleocapsid. The nucleocapsid gets the viral genome where it is supposed to be, then releases it into the cytoplasm. RNA viruses remain in the cytoplasm; DNA viruses go to the nucleus.

VIRUS	VAP	RECEPTOR	CELL
EBV	gp350	Complement Receptor 2 CR2 = CD21	B cells
B19	n/a	Erythrocyte P antigen	Erythrocyte precursors
HIV	gp120	CD4 CXCR5, CCRC4	T helper cells
Influenza	HA gp	Sialic acid	Epithelial cells

Table 2.1: Attachment
This figure is meant to serve as a visual guide through the next several pages. While there are many steps involved in the viral life cycle, and a dissimilarities between enveloped and naked viruses, the general theme remains the same—get in, get it done, get out. That is, the virus needs to get into the host cell (get in), manipulate that cell to replicate the viral genome, produce virion proteins, and in most cases, more complex viral proteins (get it done), and then eventually leave to infect the next cell (get out).

"Get it done" means **mRNA synthesis** (5), **translation** (6), **and replication** (7). This is the most varied process by virus and virus type. The genome is useless unless it can be transcribed into functional mRNAs capable of binding to ribosomes and of being translated into proteins. The means by which each virus accomplishes these steps depend on the structure of the genome and the site of replication. We explore DNA, ss(+)RNA, and ss(−)RNA in the next section, and save HIV for its own lesson. RNA viruses are read by ribosomes and translated. There aren't mechanisms to control which "genes" get translated and how often. If a start codon is available, the ribosome binds and starts translation. Which means that RNA viruses cannot regulate which proteins get coded. DNA viruses, on the other hand, can take advantage of transcription factors, making viral genes more likely to be transcribed, but also controlling the timeframe in which they are transcribed. DNA viruses are sophisticated, and a virus replication cycle takes on a phased approach, as discussed below. Regardless of how complicated it might be, all viruses build a **viral genome** and **structural proteins**, while the cell's own processes are slowed down.

"Get out" means **assembly** (8), **and release** (9) through either lysis or exocytosis.

The host cell is cranking out new virus and new viral proteins. The process of **assembly** begins when the necessary pieces have been synthesized, and the concentration of the structural proteins reaches a level that drives the process thermodynamically, much like a crystallization reaction. The assembly process combines the **genome** and the **capsid**. Then all the genome-filled capsids need to leave.

Enveloped viruses got in by fusing a stolen plasma membrane with this cell's plasma membrane. Enveloped viruses leave by stealing this cell's plasma membrane, and will use it for the next attachment and penetration. During viral protein synthesis, **glycoproteins** were being constructed in the RER and processed by the Golgi. Vesicles carry these glycoproteins to the host cell's membrane. Then the virus **buds off like a vesicle**. Only if it is wearing the plasma membrane (envelope) of its former host cell will it be able to fuse into the next cell. Because budding off like a vesicle does not compromise the host cell's membrane, the leaving of an enveloped virus need not kill the host cell.

Naked viruses can't use this budding to get out, just like they couldn't use fusion to get in. Without a plasma membrane to cloak itself in, naked viruses can leave the host cell in two ways: either through **exocytosis**, riding in a vesicle to the plasma membrane (this requires the virus be replicated and assembled within a vesicle), or by poking a hole in the plasma membrane on its way out, called **lysis**. Most naked viruses leave through lysis. Lysis involves the compromise of the plasma membrane, which kills the cell.

DNA Virus Infection and Replication

The cell's machinery for transcription of DNA to mRNA and for replication of DNA is found in the nucleus. All DNA viruses are double stranded (except parvovirus), and all DNA viruses locate themselves in the nucleus (except parvovirus). DNA viruses act like host DNA, and hijack host machinery. The DNA viruses get **transcribed piecemeal**, using helicase, primase, and DNA-dependent RNA polymerase, just like host DNA. The RNA that gets transcribed undergoes co- and post-translational modification to become mRNA. This virally coded mRNA exits the nucleus. To the rest of the cell, the virus-coded mRNA is indistinguishable from host-coded mRNA.

The cell does the work it usually does. Ribosomes **translate** the mRNA into amino acids. Signal sequences ensure that the proteins are made where they are supposed to be—either in a plasma membrane or in the cytoplasm. Chaperones make sure the virus-coded proteins are folded correctly. Complex viruses even transcribe transcription factors, ensuring that the right proteins are transcribed at the right time.

Universally, a virus hijacks host machinery. DNA viruses hijack host RNA polymerase to make mRNA for host cell ribosomes to work on. What those ribosomes make is virus-encoded proteins. Because DNA viruses can be transcribed piecemeal, DNA viruses can harness the power of transcription factors. This allows DNA viruses to segment their infectious cycle into three phases: immediate, early, and late. The **immediate early genes** code for **transcription factors.** These transcription factors facilitate transcription of the early genes. **Early genes** code for proteins that **inhibit host function**, slowing down transcription and translation of host mRNA, redirecting nucleotides, ribosomes, and amino acids to viral tasks. In the beginning it is about subduing the victim. Once subdued, **replication of the viral genome** becomes the focus. Subduing the host and replication of genome is what early transcription genes are about. As those viral genomes are replicated, the victim depleted of its resources, there is again a switch. As viral genome accumulates, late gene transcription factors also accumulate. **Late transcription** genes are about **making the structural proteins** of the **capsid**. Seeing the resources drying up, the virus assembles for departure.

DNA viruses use host DdDp and DdRp. Most DNA viruses transcribe their own DdDp as well. The viral-coded versions are generally faster, serving to replicate more viral genome, but they are also sloppier, making mistakes in that genome. This is one method of genetic variation that allows viruses to evade host defenses. Not only do these viruses not wait for the cell to divide by making viral DdDp (the only time host DdDp would have any activity), they also ensure antigenic variation to evade the immune system using that viral DdDp.

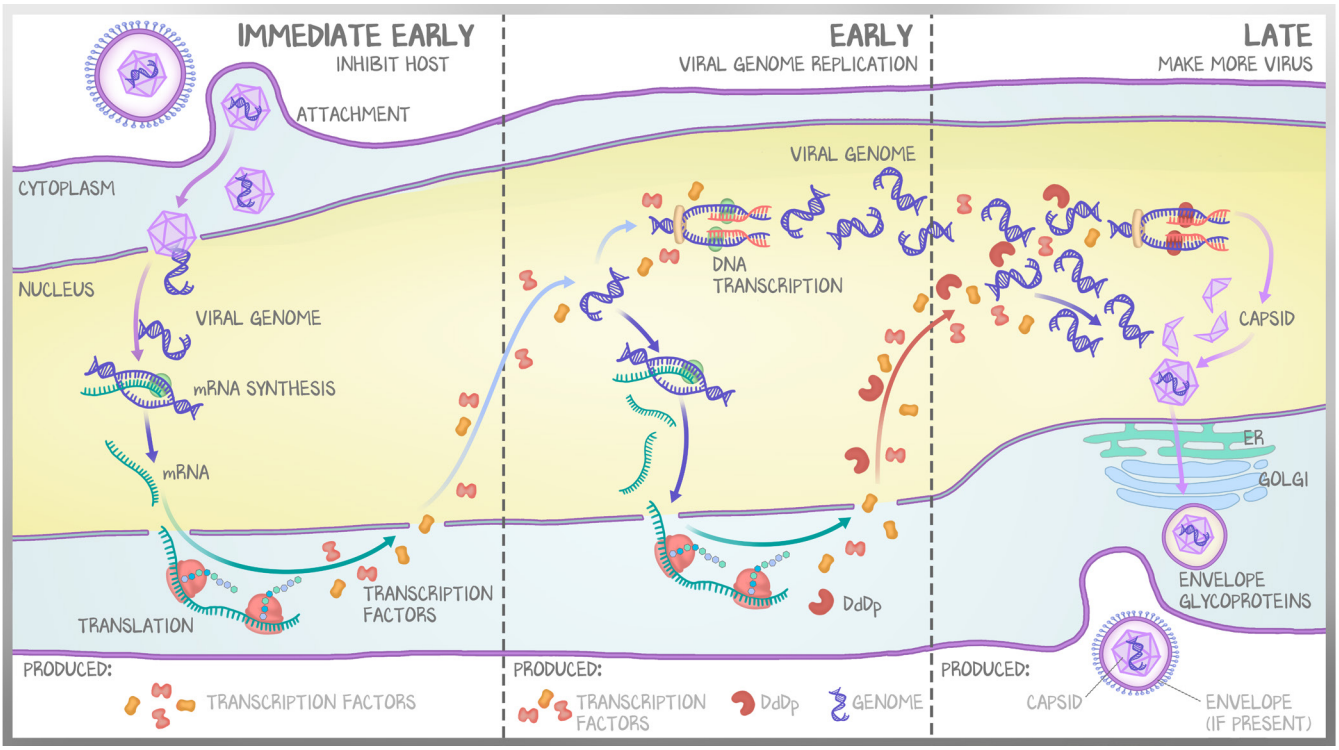

Figure 2.2: DNA Viral Replication
DNA viruses do their work from within the nucleus. Because they are double-stranded DNA, they can use transcription factors to orient and time which of their genes get transcribed when. The immediate early genes code for mRNA that codes for proteins that are transcription factors for viral DNA promoters. These transcription factors ensure that nucleotides and host RNA polymerase are dedicated to early genes. Early genes transcribe mRNA that transcribes proteins that replicate the genome. Once a critical level of genome is made, transcription factors switch to late genes. Late genes code for capsid proteins and assembly proteins, preparing for the new viruses' exit.

Positive-Sense RNA Virus Replication

Positive-sense RNA viruses means, literally, that they "**make sense**" to a ribosome. Ribosomes read mRNA from the nucleus 5' to 3'. Positive-sense RNA viruses have their entire genome oriented in a way the ribosome can understand.

RNA viruses are always single stranded (except parvovirus). RNA viruses replicate and transcribe in the cytoplasm. Because RNA viruses use RNA as their template, RNA-dependent RNA polymerase will make double-stranded RNA. Eukaryotic cells never have double-stranded RNA. The cell tends to notice RNA viruses in the cytoplasm. To combat host defenses, RNA viruses often encode for proteins that limit these responses.

Because ss(+)RNA can be read by the ribosomes, **virus-encoded RNA-dependent RNA polymerase** is one of the first proteins made. RdRp then uses the viral genome to make anti-sense strands. These are complementary and antiparallel to the viral genome and act as **intermediaries**. The same RdRp that made the intermediary, then uses it as a template. The effect of RdRp on the intermediary is to make both mRNA and viral genome, since "mRNA" and viral genome are the same thing in the case of a ss(+) RNA virus.

Because they don't NEED any proteins to come with them, positive-sense RNA viruses do **not need an envelope** and so **can be naked viruses**.

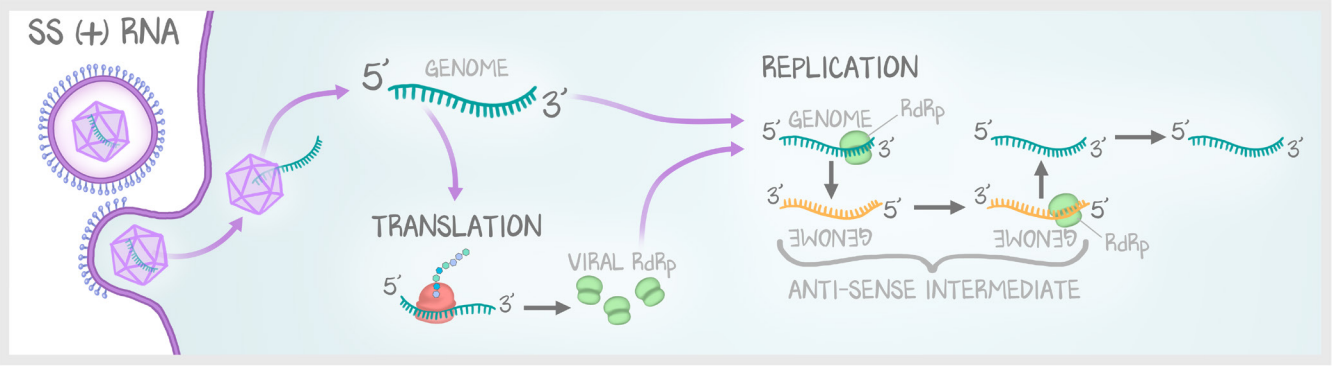

Figure 2.3: Positive-Sense RNA Replication
Positive-sense RNA viruses do NOT have to bring their own RdRp, because their genome can code for RdRp and their genome is ready to be read by ribosomes. ss(+)RNA viruses can bring their own RdRp, but don't have to. ss(+)RNA viruses must go through a negative-sense RNA intermediate, which acts as a template to make more viral genome.

Negative-Sense RNA Virus Replication

Negative-sense RNA viruses means, literally, their genome makes "**no sense**" to a ribosome. That means two things. Since no protein will be made by ribosomes from the start, negative-sense RNA viruses must **bring their own RNA-dependent RNA polymerase** with them. And if they have to bring with them more than just their capsid and genome, **all negative-sense RNA viruses must be enveloped**.

Because they bring their own RdRp, ss(−)RNA viruses begin transcribing mRNA from the viral genome. The transcribed mRNA is positive-sense RNA the ribosomes can read, but is not viral genome. The same RdRp that makes the positive-sense mRNA intermediary that ribosomes can read also uses that same positive-sense mRNA intermediary to transcribe more ss(−)RNA genome. Both ss(−)RNA viruses and ss(+)RNA viruses use RdRp to replicate genome from a template intermediary. It's just the intermediary for ss(+)RNA viruses is antisense, while the intermediary for ss(−)RNA is positive sense. Because ss(−) RNA start off antisense and cannot be read by ribosomes, they are required to bring RdRp with them to make the first step, creating the positive-sense template that will be used as the template for genome and is the mRNA sequence that ribosomes can understand. Once the process is underway, once there are many mRNA strands, many genomes, and many RdRps, there is very little to separate them.

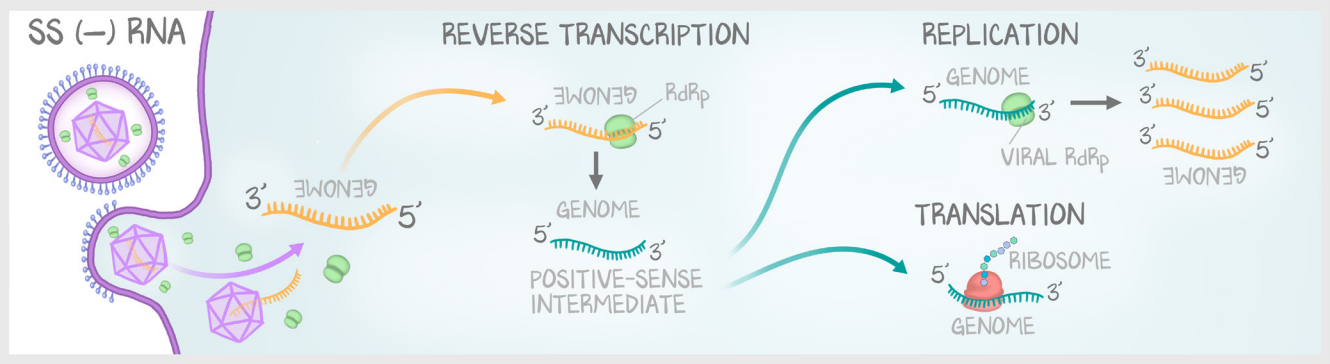

Figure 2.4: Negative-Sense RNA Replication
Negative-sense RNA viruses MUST bring their own RdRp because their genome cannot be read by ribosomes, but the mRNA they turn into makes more RdRp, and RdRp uses that mRNA strand to make more genome.

Host Cell Outcome

There are four responses that could occur at the cellular level: failed infection, cytolytic infection (cell dies), a persistent infection (replication without cell death), or a latent infection (presence of virus without virus production but with the potential for reactivation). We look at each in detail. **Viral load** is a measure of how many viruses (usually reported in particles per milliliter of plasma) are in a person, a quantification of the viral burden.

Cytolytic infections kill the host cell. Less subtle viruses are the cause of cytolytic infections. Killing the host is short-sighted, usually getting more resources immediately but compromising long-term survival. A number of events can trigger the loss of the host cell. Viral antigen expression on plasma membranes (the MHC-1 mechanism) does not give the all-clear signal to CD8 cells, who express chemokines that induce apoptosis. DNA damage may also signal apoptosis. DNA damage can occur when viruses degrade the host genome to access more nucleotides. But the most common cause of a cell host's death is **naked capsids' leaving**. If there is an envelope, then there need not be a penetration of the cell's plasma membrane. The budding off of envelopes means there is at least the potential to be nonlytic (plenty of lytic viruses are enveloped, however). But naked virions must get out of a cell. When they do, they puncture holes in the plasma membrane, causing cell lysis. If the viral replication kills the host cells, it limits itself to causing **acute illnesses**. The naked virions that are either ss(+)RNA or DNA (negative-sense RNA are all enveloped) tend to cause **acute infections only**. These show a rise in viral load, which coincides with the onset of symptoms; then viral load decreases with the relief of symptoms, never to return again.

Acute infections are things like the common cold (rhinovirus, coronavirus), diarrhea (norovirus, rotavirus), or hepatitis A infection.

Latent infection is when a virus stops replicating, the cell and the immune system unaware of its presence. The first infection presents with acute disease—viral load rises, symptoms are felt; viral load fall, symptoms resolve. But then it goes dormant, present in a cell but without replication. Without replication, without active viral load, no symptoms are felt. Then, later, under periods of stress, the virus **reactivates**. The virus replicates again. Viruses that cause latent infections cause an **acute illness again** with **rapid replication** and a peaking of viral load, coinciding with symptoms—just like the original acute infection. The best example of this is herpesviruses. An initial infection with varicella-zoster causes chickenpox. The primary viremia induces systemic widespread lesions. The primary infection is acute—short-lived by intensely painful vesicles. The immune system develops antibodies to varicella-zoster. But the virus hides in the dorsal root ganglion of sensory nerve fibers. The virus got

everywhere; every sensory nerve has virus in it. But the virus is not replicating. Decades later, during periods of immunocompromise, the virus reactivates, and starts replicating in one neuron. The neuron it is replicating in will die. Every other neuron remains with dormant virus. But this neuron, before it dies, permits the virus to travel to the skin that nerve innervates. Shingles is the virus inducing an acute, lytic infection, of the skin cells the nerve innervated. The virus is dormant in sensory nerves of all of the dermatomal distribution, but happens to reactivate only in one.

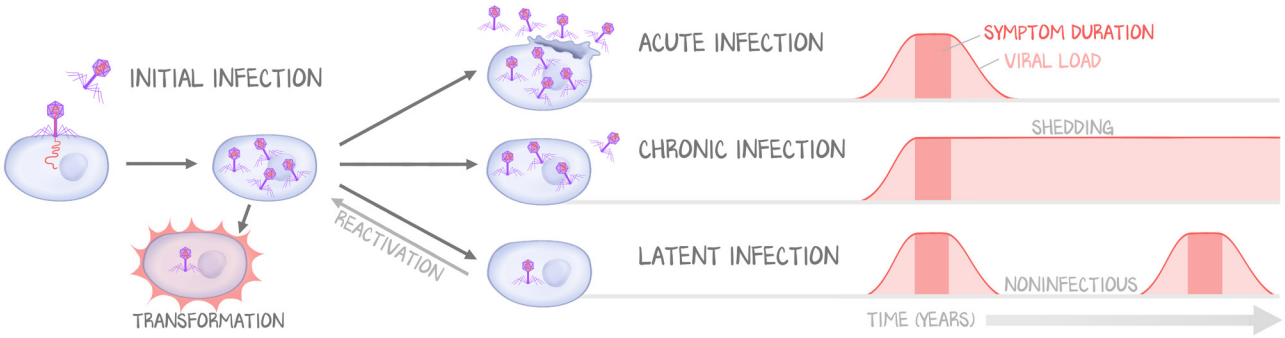

Figure 2.5: Diagram of Clinical Courses and Host Cell Outcomes
The line represents the amount of detectable virus in tissue (though it usually also means in blood), while the mark represents active acute symptoms. Host cells can either succumb to infection and die (often as a result of lysis); persist with chronic infection, shedding virus; go latent with undetectable levels for a period of time; or become transformed by the infection into malignancy. Also demonstrated is the slow progression to active infection.

All host cell outcomes from here down are **nonlytic**, and therefore ensure survival of the host cell. There are several variations on what can happen following infection with a virus that does not kill the host cell.

Persistent infection is synonymous with **chronic infection**, and describes an infection with a virus that is always replicating, but at a slow, smoldering rate. Persistent infection will show a rise in the viral load, which will **plateau.** The virus never goes dormant, so the effects of viral replication are present, but in such a small amount the patient is unaware. Chronic infections of hepatitis C, for example, show no outward signs until the patient develops cirrhosis. The cirrhosis is a product of ongoing inflammation, the immune system aware and fighting the hepatitis C, but the patient unaware of their infection. The main difference between persistent infections and latent infections is that the persistent infections never stop replicating and the virus is never cleared from the blood, while latent infections' dormant periods have no viral replication. Both are "chronic infections" (the virus is present for a long time), and so we prefer the use of persistent over chronic.

Transformation of host DNA can cause insertion of new oncogenes, activation of oncogenes, and suppression of tumor suppressors, which then leads to unregulated cell growth and eventual malignancy. This is separate from chronic inflammation leading to oncogenesis, as in hepatitis C and B causing cirrhosis and hepatocellular carcinoma. Some viruses have direct impact on cell cycle regulators. In order to interfere with host DNA, the virus must be DNA or a retrovirus. For example, some **HPV strains** (6 and 11) can cause warts (unregulated growth), while **HPV strains** 16 and 18 cause cervical cancer (unregulated growth via E6 and E7, and malignant transformation via inhibition of p53 and Rb). **HHV8** is a DNA virus associated with malignant transformation into Kaposi's sarcoma, which accompanies AIDS. **HLTV-1** causes leukemia.